

Continuation of USSN 09/625,406

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AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph appearing at page 1, lines 4-6 with the following amended paragraph:

This application is a continuation of U.S. patent application serial number 09/625,406, filed July 25, 2000, which is a continuation of U.S. patent application serial number 08/941,445, filed September 30, 1997 and now U.S. Pat. No. 6,107,060, which claims priority to provisional patent application serial No. number 60/026,855 filed September 30, 1996. Said provisional application is incorporated herein by reference to the extent not inconsistent herewith.

Please replace the paragraph appearing at page 2, lines 15-22 with the following amended paragraph:

The use of cDNA clones of plant soluble starch synthases has been reported. The amino acid sequences of pea soluble starch synthase isoforms I and II were published by Dry et al. (1991, Plant Journal, 2:193-202). The amino acid sequence of rice soluble starch synthase (SSTS) was described by Baba et al. (1993, Plant Physiology,). This last sequence (rice SSTA) incorrectly cites the N-terminal sequence and hence is misleading. Presumably this is because of some extraction error involving a protease degradation or other inherent instability in the extracted enzyme. The correct N-terminal sequence (starting with AELSR (SEQ. ID NO:38) is present in what they refer to as the transit peptide sequence of the rice SSTS.

Please replace the paragraph appearing at page 3, line 21 to page 4, line 8 with the following amended paragraph:

The other STS enzymes have become known as soluble starch synthases, following the pioneering work of Frydman and Cardini (Frydman and Cardini, 1964, Biochem. Biophys. Res. Communications 17: 407-411). Recently, the appropriateness of the term "soluble" has become questionable in light of

discoveries that these enzymes are associated with the granule as well as being present in the soluble phase (Denyer et al., 1993, *Plant J.* 4: 191-198; Denyer et al., 1995, *Planta* 97: 57-62; Mu-Forster et al., 1996, *Plant Physiol.* 111: 821-829). It is generally believed that the biosynthesis of amylopectin involves the interaction of soluble starch synthases and starch branching enzymes. Different isoforms of soluble starch synthase have been identified and cloned in pea (Denyer and Smith, 1992, *Planta* 186: 609-617; Dry et al., 1992, *Plant Journal*, 2: 193-202), potato (Edwards et al., 1995, *Plant Physiol.* 112: 89-97; Marshall et al., 1996, *Plant Cell* 8: 1121-1135) and in rice (Baba et al., 1993, *Plant Physiol.* 103: 565-573), while barley appears to contain multiple isoforms, some of which are associated with starch branching enzyme (Tyynela and Schulman, 1994, *Physiol. Plantarum* 89: 835-841). A common characteristic of STS clones is the presence of a KXGGLGDV (SEQ. ID NO:39) consensus sequence which is believed to be the ADP-Glc binding site of the enzyme (Furukawa et al., 1990, *J. Biol. Chem.* 265: 2086-2090; Furukawa et al., 1993, *J. Biol. Chem.* 268:23837-23842).

Please replace the paragraph appearing at page 4, lines 24-26 with the following amended paragraph:

Plant starch synthase (and *E. coli* glycogen synthase) sequences include the sequence KTGGL (SEQ. ID NO:40) which is known to be the ADPG binding domain. The genes for any such starch synthase protein may be used in constructs according to this invention.

Please replace the paragraph appearing at page 5, line 26 to page 6, line 4 with the following amended paragraph:

Another use is to recover one of the polypeptides of the hybrid polypeptide. Chemical and biological methods are known for cleaving the fused peptide. Low pH can be used to cleave the peptides if an acid-labile aspartyl aspartate-proline linkage is employed between the peptides and the peptides are

not affected by the acid. Hormones have been cleaved with cyanobromide. Additionally, cleavage by site-specific proteolysis has been reported. Other methods of protein purification such as ion chromatography have been enhanced with the use of polyarginine tails which increase the overall basicity of the protein thus enhancing binding to ion exchange columns.

Please replace the paragraph appearing at page 9, lines 17-25 with the following amended paragraph:

By the methods of this invention, transformed cells are produced comprising the recombinant nucleic acid molecules capable of expressing the hybrid polypeptides of this invention. These may be prokaryotic or eukaryotic cells from ~~one-celled~~ unicellular organisms, plants or animals. They may be bacterial cells from which the hybrid polypeptide may be harvested. Or, they may be plant cells which may be regenerated into plants from which the hybrid polypeptide may be harvested, or, such plant cells may be regenerated into fertile plants with seeds containing the nucleic acids encoding the hybrid polypeptide. In a preferred embodiment, such seeds contain modified starch comprising the payload polypeptide.

Please replace the paragraph appearing at page 17, line 19 to page 18, line 4 with the following amended paragraph:

Once the ligated DNA which encodes the hybrid polypeptide is formed, then cloning vectors or plasmids are prepared which are capable of transferring the DNA to a host for expressing the hybrid polypeptides. The recombinant nucleic acid sequence of this invention is inserted into a convenient cloning vector or plasmid. For the present invention the preferred host is a starch granule-producing host. However, bacterial hosts can also be employed. Especially useful are bacterial hosts that have been transformed to contain some or all of the starch-synthesizing genes of a plant. The ordinarily skilled person in the art

understands that the plasmid is tailored to the host. For example, in a bacterial host transcriptional regulatory promoters include lac, TAC (a functional hybrid derived from the TRP and lac promoters), trp and the like. Additionally, DNA coding for a transit peptide most likely would not be used and a secretory leader that is upstream from the structural gene may be used to get the polypeptide into the medium. Alternatively, the product is retained in the host and the host is lysed and the product isolated and purified by starch extraction methods or by binding the material to a starch matrix (or a starch-like matrix such as amylose or amylopectin, glycogen or the like) to extract the product.

Please replace the paragraph appearing at page 18, lines 5-13 with the following amended paragraph:

The preferred host is a plant and thus the preferred plasmid is adapted to be useful in a plant. The plasmid should contain a promoter, preferably a promoter adapted to target the expression of the protein in the starch-containing tissue of the plant. The promoter may be specific for various tissues such as seeds, roots, tubers and the like; or, it can be a constitutive promoter for gene expression throughout the tissues of the plant. Well-known promoters include the 10 kD zein (maize) promoter, the CAB (chlorophyll a/b binding protein) promoter, patatin patatin, 35S and 19S cauliflower mosaic virus promoters (very useful in dicots), the polyubiquitin promoter (useful in monocots) and enhancements and modifications thereof known to the art.